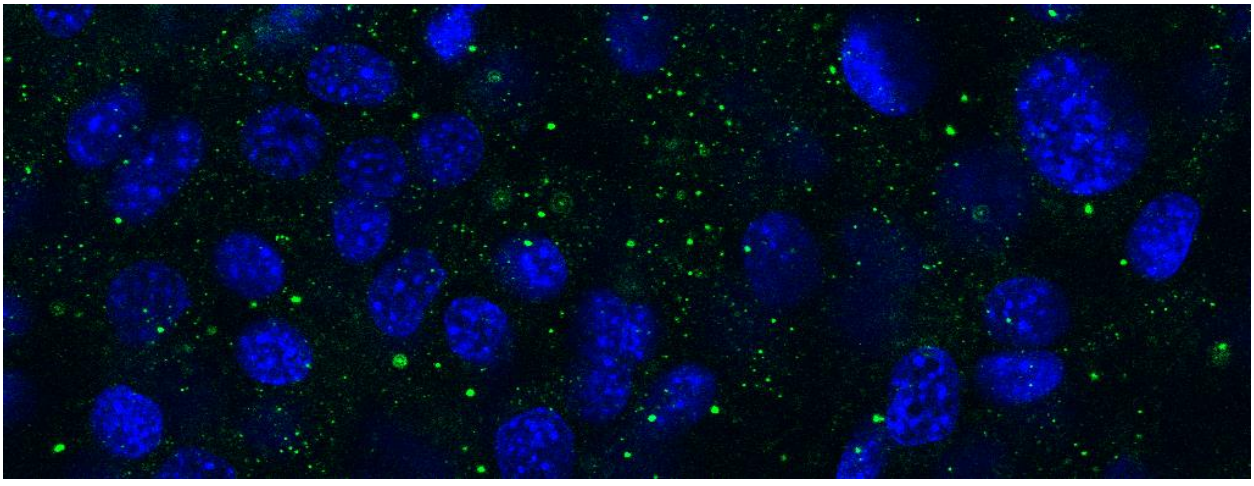
Lethbridge iGEM Collegiate 2014 Notebook – CELL CULTURE (October)

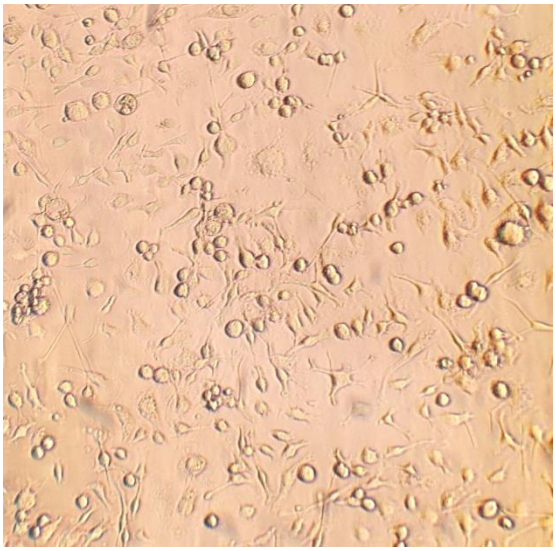
NOTE: For detailed protocols regarding starting/passaging/freezing/lipofecting cell cultures and preparing media, see "Cell Culture Protocols" document.

October 1, 2014

- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 culture.
- Added 10mL media to each of the LADMAC cultures.
- Froze two plates of HEK-293 cells.
- Split one HEK-293 plate culture into two plate cultures and one slide culture.
- To test anti-GFAP antibody, did immunocytochemistry on non-lipofected C8-D30 slide culture:
    - Aspirated media and rinsed each chamber with 1x PBS.
    - Fixed with 4% PFA for 20 minutes.
    - Rinsed twice with 1x PBS.
    - Blocked with blocking buffer plus serum for 45 minutes at room temperature.
    - Labeled with rabbit anti-GFAP (1:100 in half of chambers and 1:500 in other half in blocking buffer) for 1hr at room temperature.
    - Rinsed twice with 1x PBS.
    - Labeled half of chambers with anti-rabbit Alexa Fluor 647 (1:200 or 1:400 in blocking buffer) and half with anti-rabbit Alexa Fluor 488 (1:200 or 1:400 in blocking buffer) for 1hr at room temperature.
    - Rinsed twice with 1x PBS.
    - Coverslipped with Vectashield plus DAPI and sealed slide with nailpolish.
- Imaged C8-D30 stained slide on Olympus FluoView FV1000 laser scanning confocal microscope. There was poor staining and high background – primary antibody does not appear to work (at least not with this protocol).



No GFAP staining is evident in astrocyte cell culture but high background may be attributed to non-specific antibody binding.



EOC 13.31 (mouse microglia) cell culture as seen under a light microscope.

October 2, 2014

- Changed media in HEK-293 cultures.

October 3, 2014

- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 culture.

October 6, 2014

- Split two HEK-293 plate cultures to six plate cultures.
- Changed media in remaining HEK-293 culture.
- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 culture.
- Centrifuged, filtered and froze stocks of LADMAC conditioned media. Resuspended cell pellets and replated in fresh media.

October 7, 2014

- Split EOC 13.31 plate culture to three plate cultures.
- Changed media HEK-293 cultures.

October 8, 2014

- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 cultures.
- Added additional 10mL media to each of the LADMAC cultures.

- Lipofected non-transfected control chambers of C8-D30 slide culture with Lipofectamine 2000 (as per manufacturer's protocol but with reduced 4hr incubation with serum-free media):
    - 1 chamber: No lipofection control plus 1.6ug pcDNA3.0-NeuroD1 in media
    - 1 chamber: pcDNA3.0-NeuroD1 (0.8ug DNA per chamber)
- Lipofected one HEK-293 plate culture with 150ul DNAfectamine (as per manufacturer's protocol) and 6ug of pcDNA3.0-Lamp2B-Clover for exosome isolation.

October 9, 2014

- Changed media in HEK-293 cultures.

October 10, 2014

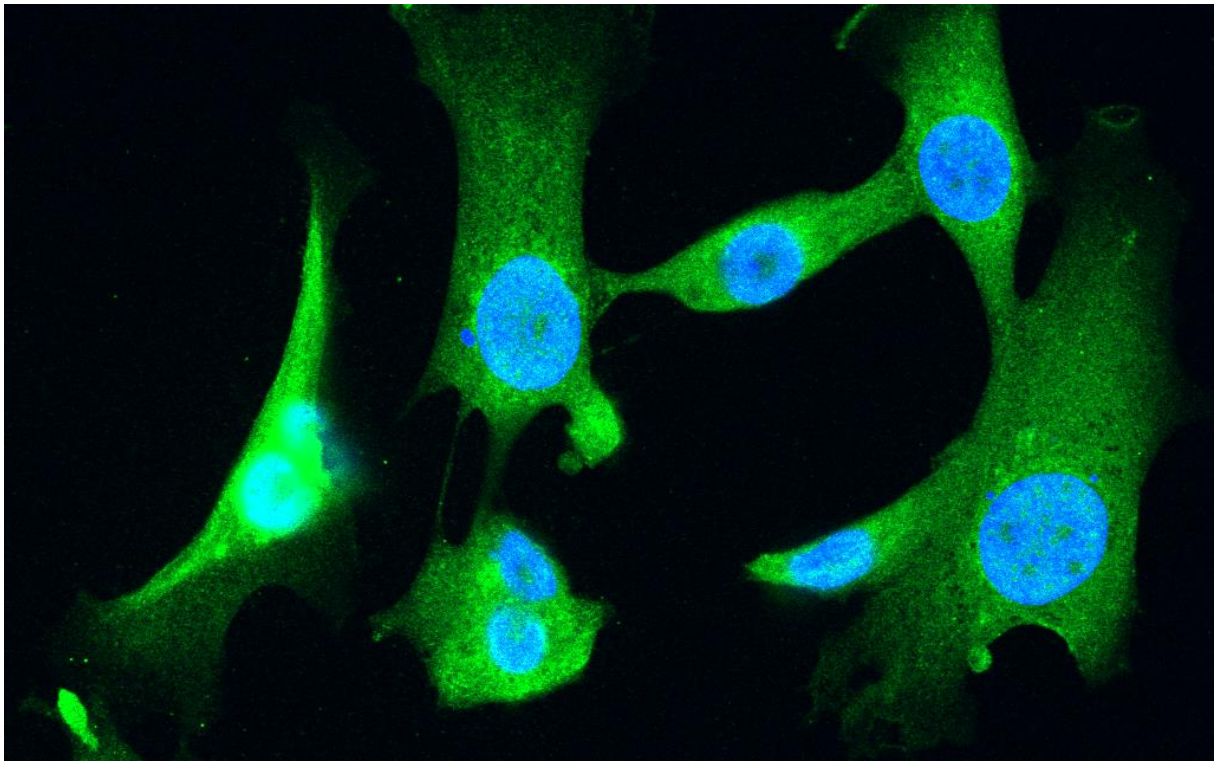
- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 cultures.

October 14, 2014

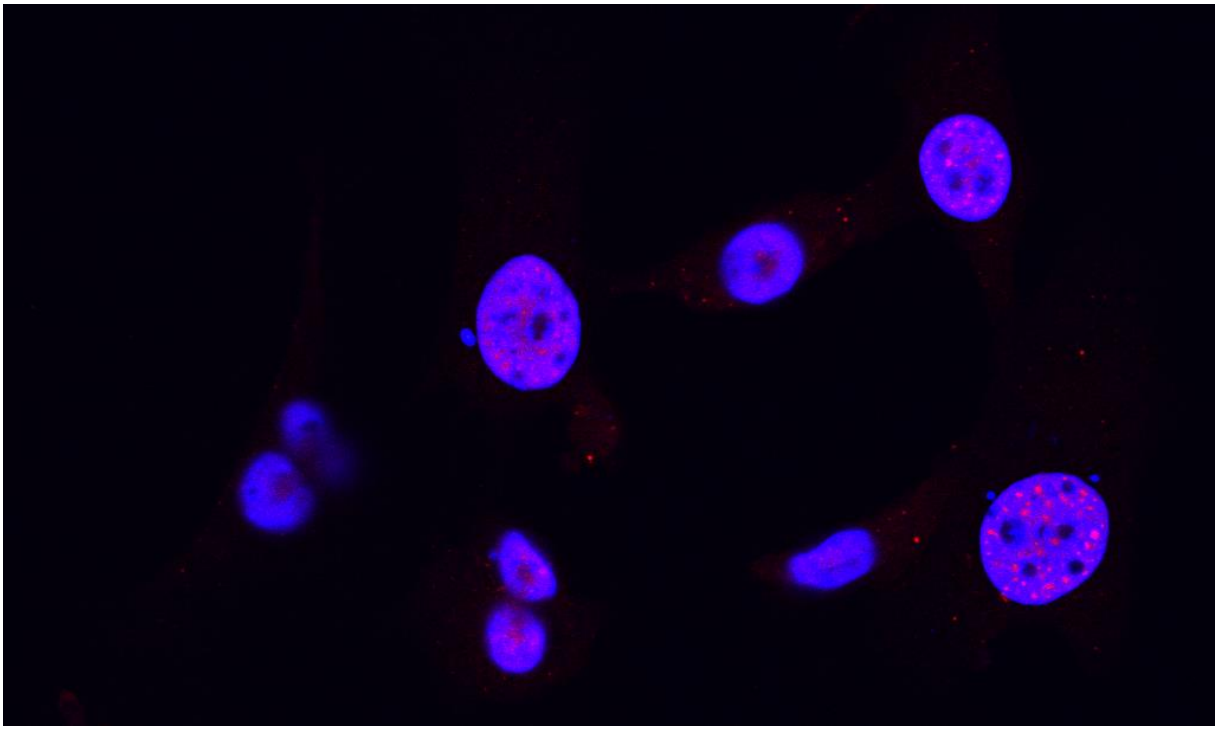
- Changed media in HEK-293 cultures.
- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 cultures.
- Split EOC 13.31 plate culture to two plate cultures and one slide culture.
- Split C8-D30 plate culture to two plate cultures and one slide culture.

October 15, 2014

- Changed media in recently split C8-D30 cultures.
- Changed media in recently split EOC 13.31 cultures.
- Did immunocytochemistry on NeuroD1-lipofected C8-D30 slide culture:
    - Aspirated media and rinsed each chamber with 1x PBS.
    - Fixed with 4% PFA for 20 minutes.
    - Rinsed twice with 1x PBS.
    - Blocked with blocking buffer plus serum for 45 minutes at room temperature.
    - Labeled with NeuN-Cy3 (1:100 in blocking buffer + 0.3% Triton-X) and Doublecortin (1:100 in blocking buffer + 0.3% Triton-X) for 3.5hr at room temperature.
    - Rinsed twice with 1x PBS.
    - Labeled with anti-goat Alexa Fluor 488 (1:200 in blocking buffer + 0.3% Triton-X) for 1.5hrs at room temperature.
    - Rinsed twice with 1x PBS.
    - Coverslipped with Vectashield plus DAPI and sealed slide with nailpolish.
- Imaged C8-D30 stained slide on Olympus FluoView FV1000 laser scanning confocal microscope.



Astrocytes expressing NeuroD1 plasmid demonstrate high Clover expression.



Astrocytes expressing NeuroD1 for one week demonstrate weak NeuN labeling (indicator of mature neurons).

October 16, 2014

- Added 7ug pcDNA3.0-Lamp2B-Clover plasmid to one EOC 13.31 plate culture (without lipofection).
- Tested lipofection conditions (with various amounts of Lipofectamine 2000 and 0.5ug pcDNA3.0-Lamp2B-Clover plasmid) in C8-D30 slide culture (one row each with 4hr incubation in serum-free media or complete media).

October 17, 2014

- Changed media in HEK-293 cultures.
- Changed media in C8-D30 cultures.
- Changed media in EOC 13.31 cultures.
- Centrifuged, filtered and froze stocks of LADMAC conditioned media. Resuspended cell pellets and replated in fresh media.